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# The incudomalleolar articulation in down syndrome (trisomy 21): a temporal bone study

Fausch, Christian ; Röösli, Christof

Abstract: HYPOTHESIS: One reason for conductive hearing loss (HL) in patients with Down syndrome (DS) is structural anomalies in the incudomalleolar joint (IMJ) that impair sound transmission. BACKGROUND: The majority of hearing losses in patients with DS are conductive. One reason is the high incidence of inflammatory processes such as otitis media. However, in some patients, the middle ear seems to be normal. The assumption of structural disorders causing a HL is supported by a previous study revealing structural abnormalities of the incudostapedial joint (ISJ) in these patients. METHODS: In a retrospective analysis, histologic sections of the IMJ of 16 patients with DS were compared with 24 age- and sex-matched subjects with normal middle ear ossicles. The length of 8 parameters of the IMJ were measured at 3 positions and compared between the 2 groups. RESULTS: Age ($p = 0.318$) and sex distribution ($p = 1$) for the DS group and the matched controls were comparable. The IMJs ($p < 0.001$) and the cartilage of patients with DS are significantly wider in most measurements compared with controls. However, the joint space is not significantly different in the 2 groups. CONCLUSION: Conductive HL might be caused by a significantly wider IMJ in patients with DS supporting the findings of a previous study reporting similar findings for the ISJ. The etiology of these findings is unclear. Patients with DS have a high prevalence of deficient collagen synthesis. Immunohistochemical analysis may be needed to investigate the collagen structure of the ISJ and IMJ in patients with DS.

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# The Incudomalleolar Articulation in Down Syndrome (Trisomy 21): A Temporal Bone Study

Christian Fausch and Christof Röösli

*Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Zurich, Zurich, Switzerland*

**Hypothesis:** One reason for conductive hearing loss (HL) in patients with Down syndrome (DS) is structural anomalies in the incudomalleolar joint (IMJ) that impair sound transmission.
**Background:** The majority of hearing losses in patients with DS are conductive. One reason is the high incidence of inflammatory processes such as otitis media. However, in some patients, the middle ear seems to be normal. The assumption of structural disorders causing a HL is supported by a previous study revealing structural abnormalities of the incudostapedial joint (ISJ) in these patients.
**Methods:** In a retrospective analysis, histologic sections of the IMJ of 16 patients with DS were compared with 24 age- and sex-matched subjects with normal middle ear ossicles. The length of 8 parameters of the IMJ were measured at 3 positions and compared between the 2 groups.
**Results:** Age ($p = 0.318$) and sex distribution ($p = 1$) for the DS group and the matched controls were comparable. The IMJs ($p < 0.001$) and the cartilage of patients with DS are significantly wider in most measurements compared with controls. However, the joint space is not significantly different in the 2 groups.
**Conclusion:** Conductive HL might be caused by a significantly wider IMJ in patients with DS supporting the findings of a previous study reporting similar findings for the ISJ. The etiology of these findings is unclear. Patients with DS have a high prevalence of deficient collagen synthesis. Immunohistochemical analysis may be needed to investigate the collagen structure of the ISJ and IMJ in patients with DS. **Key Words:** Conductive hearing loss—Down syndrome—Histopathology—Incudomalleolar joint—Temporal bone.
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After early efforts by Seguin to differentiate the symptoms of trisomy 21 (Down syndrome, DS) from cretinism, the English neurologist John Landon-Down first described a specific nosologic category of the symptoms as a distinct form of mental disability in 1862 (1). The typical karyotype had been discovered in 1959 by Lejeune et al. (2). Down syndrome is one of the most common genetic aberrations in humans that results in intellectual disability. It is caused by a chromosomal condition in which a third copy of the long arm of human chromosome 21 is present (3). Among other disabilities, children with DS have a high prevalence of hearing loss (HL). It is estimated to be found in two-thirds of all patients. Of these, around 80% are conductive, of which, 60% are related to tympanic membrane perforation and/or otitis media with middle ear effusion. However, middle ear structures seem normal in many patients, and the cause for the conductive HL remains unclear (4).

Address correspondence and reprint requests to Christian Fausch, M.D., Department of Otorhinolaryngology, University Hospital Zurich, Frauenklinikstrasse 24, CH-8091 Zurich, Switzerland; E-mail: chfausch@gmail.com

The authors disclose no conflicts of interest.

Down syndrome is known to be associated with pathologic changes of joints such as ligament laxity, hypermobility, and bony anomalies (5). Up to 20% of patients with DS experience atlantoaxial instability (6). Therefore, it is reasonable to assume that the joints in the middle ear could also be affected by pathologic changes. In the past, most temporal bone studies of subjects with DS focused on the histopathologic characteristics of the inner ear, not placing much emphasis on the middle ear. However, some histopathologic studies of temporal bones have shown numerous abnormalities of the middle ear, especially the ossicular chain (7). A study of the incudostapedial joint (ISJ) in DS revealed that the distance between the head of the stapes and the lenticular process of the incus is significantly wider compared with age-matched normally hearing subjects. A wider joint might result in a less efficient transmission of sound energy (8). These findings indicate one possible cause for conductive HL; however, the specific role of the incudomalleolar joint (IMJ) in contributing to hearing sensitivity remains unclear. Kaf (9) compared wideband energy reflectance in patients with DS with healthy controls and found significant differences between 5.7 and 8 kHz in the DS group. This difference manifests in a higher frequency range than expected. Energy reflectance in patients with ossicular



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interruptions is reported to show sharp minima between 0.6 and 2 kHz (10). One possible explanation is the difference in ear canal volume (smaller in the DS group), another the lack of conductive hearing loss, as hearing thresholds are not reported. Many investigations have been made to determine the role of the IMJ in sound transmission. In 2002, Willi et al. (11) demonstrated that the articulation is not to be seen as a rigid body. Moreover, it seems that the elastic tissues of the medial and lateral ligaments define the mechanics of the frequency dependent motions. Laxity in this particular joint causes inappropriate motion between the malleus and incus leading to transmission loss, which affects the middle ear. Such laxity may be caused by a wider joint.

In this study, we investigated the quantitative aspects of the anatomy of the IMJ in subjects with DS to characterize further the findings of previous studies (8). We hypothesized that the histologic anatomy of the IMJ in patients with DS would differ from the IMJ of a matched control group without DS (Fig. 1).

## MATERIALS AND METHODS

The temporal bone collection of the ENT Department of the University Hospital of Zurich served as a source for the temporal bone sections. Of 19 subjects with DS, 16 met the inclusion criteria (presence of a section through the IMJ and the absence of artifacts). They were compared with 24 age- and sex-matched controls.

The temporal bones had been processed for light microscopy using the standard method of fixation in formalin, decalcification using trichloroacetic acid or ethylenediaminetetraacetic acid, embedding in celloidin, serial sectioning in the horizontal plane at a section thickness of 20 µm, and staining of every 10th section using hematoxylin and eosin (12). The histologic sections were stored in cardboard folders in a climate-controlled room.

All temporal bone slides from each subject were studied by the 2 authors using a binocular microscope (Leica DMRB; Leica Microsystems, Wetzlar, Germany) to identify the section that best represented the IMJ for both the DS and control groups. In the control group, the inclusion criterion was nonpathologic middle ear anatomy (Fig. 1). The histologic sections were digitally photographed on high resolution (3,900 × 3,900 pixels) with a camera (AxioCam; Carl Zeiss AG, Oberkochen, Germany) attached to the microscope using a C-Mount optical interface.

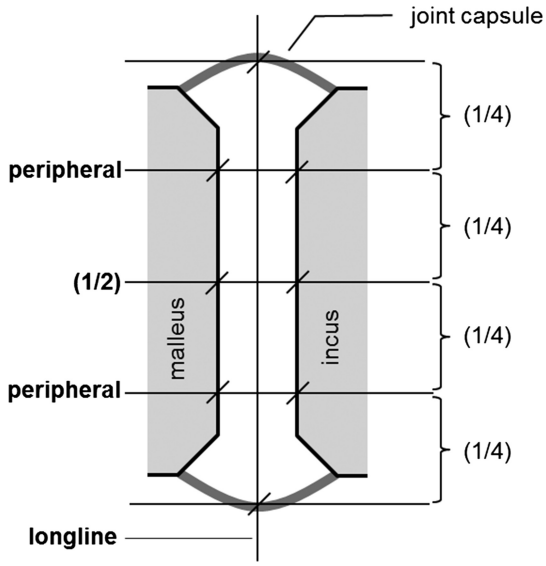


**FIG. 2.** Illustration of the measurement positions in a logic diagram of a midsection of the incudomalleolar articulation. (1/2) stands is defined as midline.

The camera was linked to a scanning program (Axiovision 4.6.3; Carl Zeiss AG, Oberkochen, Germany), which was running on a personal computer (HP Compaq dc7900 Convertible Minitower; HP, Palo Alto, USA). Investigations and the photographs were made using a magnification of ×1.6 and ×5.0.

All measurements were made using ImageJ (http://rsbweb.nih.gov/ij/). On each cross-sectional slide of the articulation, 25 parameters were defined and statistically compared between the 2 groups. The line (called *longline*) was defined as the distance between the synovial membranes resting on the lateral ligaments of the capsule in the joint line. This line served as a reference and was quartered, resulting in 3 measurement positions, as demonstrated in Figure 2. At all 3 positions, the distance between the osseous portion of the malleus and incus (b-line), the joint space between the 2 ossicles (Discus), the total cartilage of the malleus (Mall C tot), the calcified cartilage of the malleus (Mall cC) as well as the total cartilage of the incus (Inc C tot) and the calcified cartilage of the incus (Inc cC) were measured at a 90-degree angle. The hyaline cartilage is the result of the subtraction of total cartilage minus the calcified cartilage for both malleus (Mall hC) and incus (Inc hC). Summarized, the lengths of 8 parameters

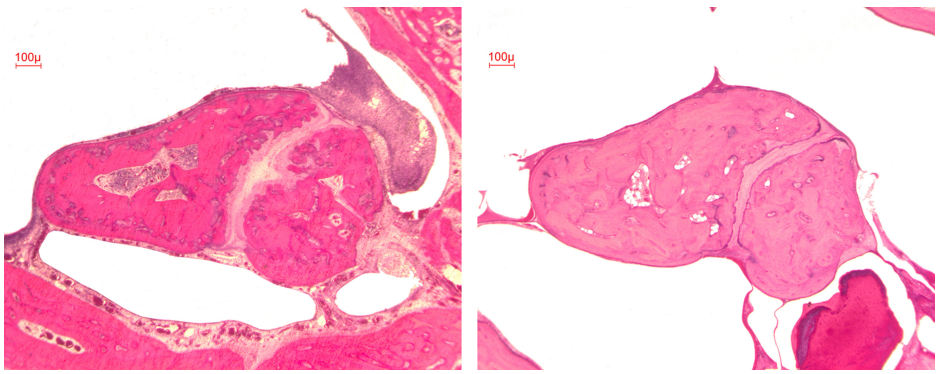


**FIG. 1.** Hematoxylin and esosin–stained histologic sections through the IM articulation, both from the right ear. On the left side, a histologic section of a DS female, 1 month. On the right side, a section of the incudomalleolar joint of a control case, 7 years.



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for each quarter line were analyzed and compared, as shown in Figure 3. Results of the measurements at the 2 peripheral positions were averaged.

The statistical evaluation was attained with an SPSS statistical software package (IBM, Chicago, IL, USA). The different parameters of the DS group were compared with the corresponding parameters of the control group using the Mann-Whitney test to find statistically significant differences. The Mann-Whitney test or the Pearson chi-square/Fisher's exact test was used to compare the 2 groups. When 1 subject had usable histologic slides on both the left and the right temporal bones, the measured results were averaged.

## RESULTS

Of the 16 subjects with the diagnosis of DS, the temporal bones from both sides were available in 7. In the control group, both sides were available in 3 subjects. To reduce biases by statistical leverage of single testing subjects with 2 temporal bones available, we averaged the measured results from the left and the right temporal bones.

Table 1 describes the details of the subjects in the 2 groups. The mean age of the control group was somewhat higher than the study group, but the difference was not statistically significant ($p = 0.318$). Regarding sex distribution, proportions were comparable with 8 female (50 %) and 8 male subjects (50 %) in the DS group and 12 female (50 %) and 12 male subjects (50 %) in the control group ($p = 1$). Twenty-five parameters of each single midsection slide were measured, analyzed, and statistically compared between the 2 groups. Using descriptive statistics, the median, mean, and standard deviation were evaluated. Statistically significant differences in the comparison of the IMJ of the DS and the control groups were found for the midline and peripheral categories as listed in Table 2 and represented graphically in Figures 4 and 5, respectively. The distance between the cortical bone of the malleus and the cortical bone of the incus was significantly wider in the DS group for the midline [b-line (1/2)] and peripheral [(peripheral) b-line] comparisons. In addition, the DS cases had significantly more cartilage covering the bone of the malleus and incus. Statistical significance was found for the total cartilage [(Mall C tot) and (Inc C tot)] and the hyaline cartilage [(Mall hC) and (Inc hC)] for both malleus and incus in the midline as well as peripheral measures.

**TABLE 1.** *Description of the 2 groups*

| | | Sex | | Age | |
|---|---|---|---|---|---|
| | n | Male n (%) | Female n (%) | Mean (mo) | Range (mo) |
| Down syndrome | 16 | 8 (50) | 8 (50) | 31.5 | 0.8–111 |
| Control | 24 | 12 (50) | 12 (50) | 43.5 | 1–108 |
| *p* | | 1.0[a] | | 0.318[b] | |

[a]Pearson chi-square.
[b]Mann-Whitney test.

## DISCUSSION

This study focused on the anatomic aspects of the IMJ of subjects with the diagnosis of DS. We hypothesized that the joint in DS would differ anatomically from the IM articulation of subjects without trisomy 21. This is the first investigation of the IMJ in cases with DS and follows the study of Ogando et al. who found a significantly larger space in the ISJ in patients with DS (8).

Our investigation of the quantitative aspects of the IMJ has demonstrated that the articulation is significantly wider compared with the control group in several measurements, including the overall joint width. Additionally, there is significantly thicker total cartilage on both the malleus and incus caused by significantly thicker hyaline cartilage on both ossicles on all measurements. The calcified cartilage was significantly thicker in most measurements.

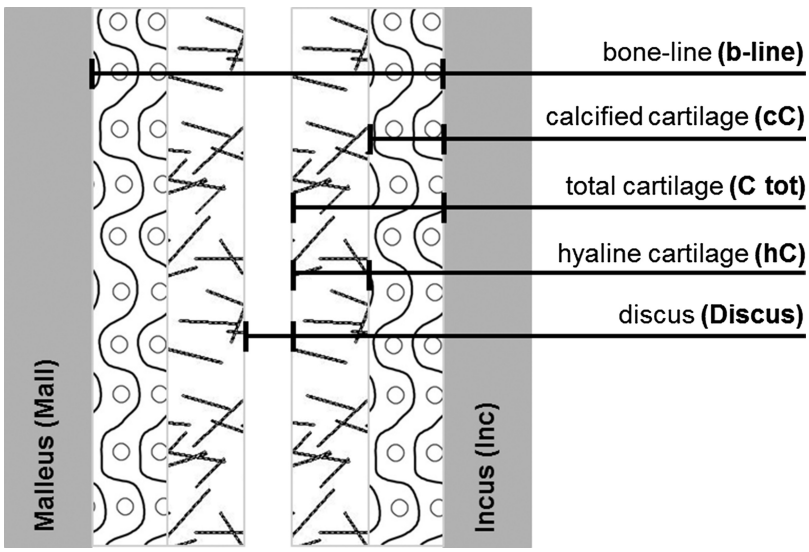


**FIG. 3.** Illustration of the measurement parameters in a logic diagram of the joint structures of the incudomalleolar joint. The parameters cC, C tot, and hC are measured for both malleus and incus.



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**TABLE 2.** *Comparison of the measurement parameters*

| Measurement position | Down syndrome mean (range) μm | Control mean (range) μm | % Difference[a] | $p^b$ |
|---|---|---|---|---|
| *SIGNIFICANT*[b] | | | | |
| (1/2) b-line | 304 (151–557) | 209 (90–363) | 31.3 | 0.003 |
| (1/2) Mall C tot | 115 (38–185) | 89 (20–232) | 22.6 | 0.048 |
| (1/2) Mall hC | 69 (15–122) | 34 (<1–96) | 50.7 | <0.001 |
| (1/2) Inc C tot | 133 (48–388) | 74 (37–143) | 44.4 | 0.002 |
| (1/2) Inc hC | 69 (10–113) | 35 (<1–69) | 49.3 | <0.001 |
| (peripheral) b-line | 326 (167–573) | 188 (115–473) | 42.3 | <0.001 |
| (peripheral) Mall C tot | 127 (68–247) | 73 (42–146) | 42.5 | <0.001 |
| (peripheral) Mall cC | 69 (19–145) | 43 (16–98) | 37.7 | 0.025 |
| (peripheral) Mall hC | 58 (35–102) | 30 (8–69) | 48.3 | <0.001 |
| (peripheral) Inc C tot | 131 (43–297) | 80 (44–139) | 38.9 | 0.001 |
| (peripheral) Inc hC | 59 (19–92) | 34 (10–84) | 42.4 | 0.003 |
| *NOT SIGNIFICANT*[b] | | | | |
| Longline | 1783 (1,501–2,474) | 1732 (1,161–2,122) | 2.9 | 0.859 |
| (1/2) Inc cC | 64 (<1–302) | 39 (<1–81) | 39.1 | 0.754 |
| (1/2) Discus | 55 (20–123) | 46 (10–101) | 16.4 | 0.521 |
| (peripheral) Inc cC | 71 (20–256) | 47 (25–81) | 33.8 | 0.090 |
| (peripheral) Discus | 67 (6–198) | 35 (2–220) | 47.8 | 0.058 |

[a]Percentage of the difference of the mean.
[b]Mann-Whitney test.

However, the joint space itself was not found to be significantly wider in the IMJ in subjects with DS compared with the control group. In summary, the IMJ is wider in patients with DS because there is more cartilage in comparison to age-matched subjects with normal middle ear anatomy. These results correlate with the findings of the quantitative analysis of the ISJ in the study of Ogando et al. They also found a wider overall joint in patients with DS. In addition, they found significantly thicker cartilage on the incus but not on the stapes in patients with DS. In our study, significantly thicker cartilage was found on incus and malleus. Ogando et al. did not differentiate between calcified and hyaline cartilage. Therefore, it remains unclear whether one specific portion of the cartilage is responsible for the differences. In contrast to our findings, Ogando et al. found a significantly wider joint space caused by a thicker articular disc in DS cases.

One difficulty in the methodology used for our study was determining the borders between the hyaline cartilage and the fibrocartilaginous tissue of the joint space. Furthermore, not all histologic cross sections were available at exactly the same location of the articulation. However, these issues only have an influence on the measurement results of the longline and only a minimal effect on the other measurement parameters that are relevant for the determination of the joint dimensions.

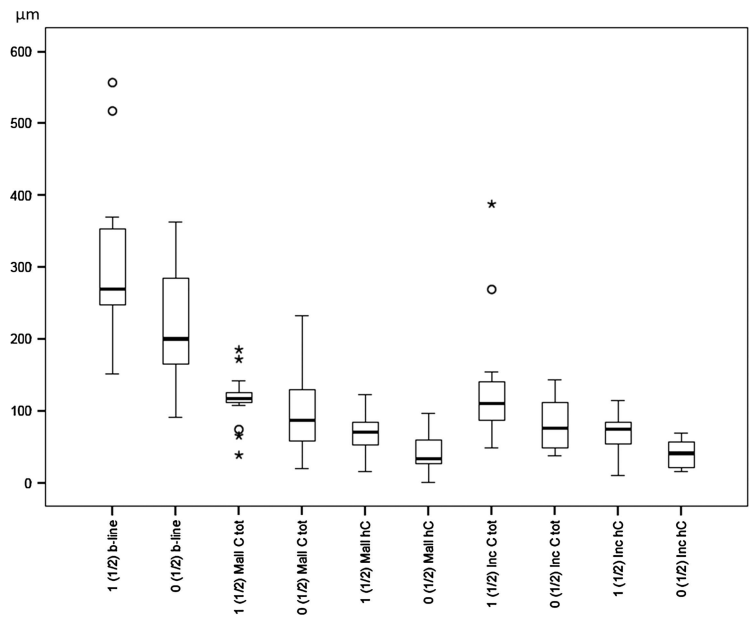


**FIG. 4.** Comparison of the parameters with statistically significant differences between the DS (1) and the control group (0) in the midline (1/2) measurement position of the incudomalleolar joint. b-line = bone-line, cC = calcified cartilage, C tot = total cartilage, hC = hyaline cartilage, Mall = Malleus, Inc = Incus.



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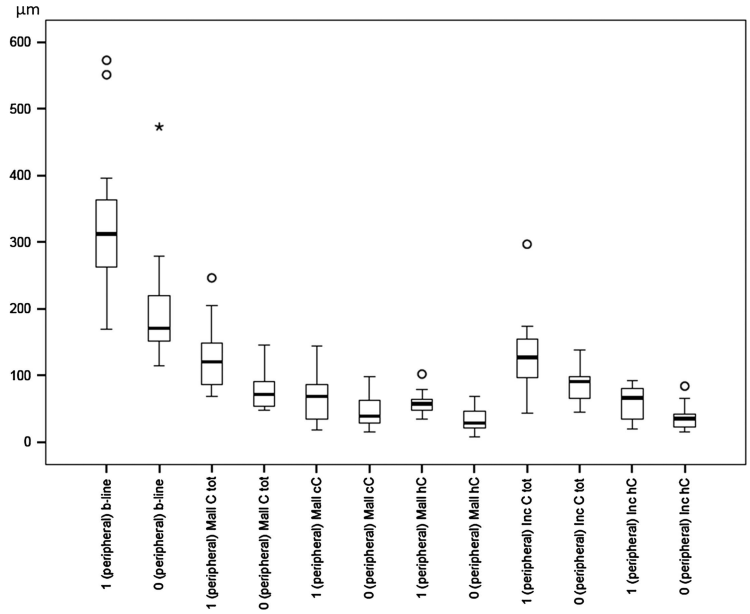


**FIG. 5.** Comparison of the parameters with statistically significant differences between the DS (1) and the control group (0) in the peripheral (peripheral) measurement position of the incudomalleolar articulation. b-line = bone-line, cC = calcified cartilage, C tot = total cartilage, hC = hyaline cartilage, Mall = Malleus, Inc = Incus.

An intact IMJ is essential for sound transmission. The results of this study show that the IMJ is significantly wider in subjects with DS compared with the IMJ in those with normal anatomy. However, the disc is not significantly different between the 2 groups, and it remains unclear whether the difference in cartilage in the IMJ is functionally significant. According to Willi et al., the mechanics of functional mobility are defined by the elastic tissues of the articulation (11). Their study on temporal bones using a laser Doppler vibrometer revealed that the relative motion between the malleus and incus at any sound pressure level can lead to a frequency-dependent sound transmission loss. Whether a wider IMJ affects sound transmission was not investigated in this study.

A correlation between hearing thresholds and the quantitative aspects of the anatomy of the IMJ is a way to estimate the effect of a large IMJ on sound transmission. Unfortunately, hearing thresholds were not available for the subjects included in this study, mainly because of lack of documentation. This is a well-known issue in temporal bone studies. Unfortunately, there is no imaging method at this time to evaluate the IMJ in vivo at this moment. The overlap in dimensions of the joint between the 2 groups indicates that not all subjects with DS have conductive hearing loss because of structural changes.

Patients with DS have a high prevalence of pathologic musculoskeletal conditions such as scoliosis, hip instability, slipped capital femoral epiphysis, patellar instability, and foot deformities (5). Collagen is a major component in musculoskeletal structures such as ligaments, tendons, cartilage, and other extracellular structures. General joint laxity in DS could be associated with deficient collagen synthesis. A previous study by Francomano et al. demonstrated that the genes that encode Type VI collagen, COL6A1 and COL6A2, are found on the most distal part of the 21st chromosome (13). Type VI collagen is a ubiquitous structural protein in the human body and a component of microfibrils in tissue (14). Disorders of these genes such as mutation or overexpression and consequently anomalies in Type VI collagen may contribute to anomalies of the middle ear joint in patients with DS. The presence of Type VI collagen in the middle ear structures of subjects with DS is not known and may be investigated with immunohistochemical methods in future studies.

Hearing impairment in patients with DS may be an additional handicap in communicative and social life. It seems that especially narrative tasks lead to difficulties for DS patients with HL (15). Hearing difficulties have negative effects not only on language learning but also on psychological and emotional progress in personal development. Early detection, diagnosis, and treatment are important to maximize the quality of life of each individual. In subjects with DS who have conductive HL with no history of otitis media or any other ossicular pathology, an abnormal IMJ and/or ISJ should be considered. These persons might benefit from middle ear exploration and ossiculoplasty.

## CONCLUSION

It could be demonstrated in this study that the IM articulation in DS differs from nonsyndromic subjects. The overall distance between the malleus and incus is significantly wider, and the hyaline cartilage is significantly thicker in subjects with DS compared with controls with



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normal middle ear anatomy. It is well known that patients with DS have a higher incidence of conductive HL because of the higher incidence of subacute and chronic otitis media leading to structural disorders of the middle ear ossicles. However, in some patients, the ossicular chain seems macroscopically normal, and structural differences of the IMJ and ISJ may apply.

If conductive HL cannot be explained by chronic otitis media, then an explorative surgical approach of the middle ear might be indicated. Such patients may benefit from ossiculoplasty, even if the ossicles seem macroscopically normal.

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